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MNase digestion for Native ChIPs

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Procedure

1. Resuspend nuclei to 5 mg/ml (based on A260 of chromatin) in Lysis Buffer + Ca.
2. Digest nuclei with 1/3 X, 1X (where X equals concentration of Mnase stock where maximal amount of di- and tri-nucleosomes occur) and 3X in separate digests, with a volume of nuclei to diluted Mnase of 10:1. Digestion is 20 min at 37° C. (I usually had 1800 ul to load on the sucrose gradient, so 600 ul of each digestion concentration).
3. Stop reaction by adding EDTA to a final concentration of 10 mM and placing on ice.
4. Spin at 2500 g for 5 min and retain released chromatin supernatants and pool (S1).
5. Resuspend nuclei pellets together in 200-500 □l of Lysis Buffer +EDTA and incubate on ice for 15 min.
6. Pass nuclei 10 times through 20 gauge needle.
7. Spin at 10,000 g for 10 min and retain supernatant (S2)
8. Removal of linker histone:
   8.1. combine S1 and S2 and raise NaCl concentration to 150 mM
   8.2. incubate on ice for 20 min
   8.3. spin at 10,000 g for 15 min and retain supernatant.
9. Supernatant was then placed drop by drop on top of 5-30% Sucrose Gradient (made using Gradient maker, per manufacturer's protocol). I always made a balance with the lower (30%) sucrose solution containing trypan blue so that after centrifugation, I could visually see if a gradient was formed.
10. Centrifuge gradient at 80,000 g for 20 hours.
11. Remove gradient very carefully from centrifuge. Take 1 mL fractions from gradient by carefully pipetting from the top.
12. Run small sample (up to 25 ul) of each fraction on an agarose gel (after ProK digestion of sample, 1 ul 30 min at 55 degrees) to determine what size nucleosomes each contains.

Solutions

Lysis Buffer	MNase Dilution Buffer
10 mM Tris pH 7.5 10 mM NaCl 3 mM MgCl2 0.4% NP-40 10 mM Na Butyrate (if studying histone acetylation) Protease Inhibitors Pre-chilled to 4° C • Lysis Buffer with 1 mM CaCl2 • Lysis buffer with 1 mM EDTA	20 mM Tris pH 8 0.25 um CaCl2 20 % Glycerol